

# Mice Lacking Endothelial Angiotensin-Converting Enzyme Have a Normal Blood Pressure

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**Abstract**—To test the hypothesis that local vascular production of angiotensin II is necessary for the normal regulation of blood pressure, we engineered a new line of genetically altered mice that lack endothelial angiotensin-converting enzyme (ACE). This was accomplished using a novel strategy of targeted homologous recombination to separate the transcriptional control of somatic ACE from its endogenous promoter and to substitute control to the albumin promoter. These new mice, termed ACE.3, do not produce ACE within the lung, the aorta, or any vascular structure. ACE activity within the kidney is only about 14% that of wild-type mice and is limited to tubular epithelium. In contrast, hepatic ACE expression in ACE.3 mice is about 87-fold that of wild-type. The blood pressure, plasma angiotensin II levels, response to ACE inhibitors, and renal function of ACE.3<sup>-/-</sup> mice are indistinguishable from littermate wild-type mice. These data show that, under basal conditions, the normal regulation of blood pressure and renal function is possible in a mouse devoid of endothelial ACE. (*Circ Res.* 2002;90:87-92.)

**Key Words:** knockout mice ■ angiotensin-converting enzyme ■ angiotensin II ■ endothelium ■ blood pressure

The renin-angiotensin system plays a central role in controlling mammalian blood pressure: mice with a genetic block of this system have systolic blood pressures 35 mm Hg lower than control animals.<sup>1-3</sup> Less clear is whether this system functions as a systemic endocrine network or whether it functions more as a series of local autocrine/paracrine networks within various organs and segments of the vasculature.<sup>4</sup> Indeed, some (including us) have hypothesized that the local production of angiotensin II by endothelial-bound angiotensin-converting enzyme (ACE) is a critical feature of normal vascular function.<sup>3,5</sup> To test the hypothesis that local vascular production of angiotensin II is necessary for the normal regulation of blood pressure, we genetically altered mice to lack endothelial ACE. This was accomplished using a novel strategy of targeted homologous recombination to separate the transcriptional control of somatic ACE from its endogenous promoter and to substitute control to the albumin promoter. These new mice, termed ACE.3, produce ACE within hepatocytes but do not make ACE within the lung, the aorta, or any vascular structure. Surprisingly, the expression of ACE by the liver is capable of substituting for endothelial ACE expression in that ACE.3<sup>-/-</sup> mice have a normal blood pressure and normal renal function.

## Materials and Methods

### Creation of Homozygous Mutant Mice

A 10.7-kb fragment of mouse genomic DNA was cloned from a mouse CC1.2 embryonic stem (ES) cell library derived from mouse

strain 129 DNA. This contained 2.4 kb of the somatic ACE promoter, the somatic ACE transcription start site, and 8.3 kb of genomic sequence encompassing somatic ACE exons 1 through 12. Using a unique *Bss*HII restriction site, a neomycin cassette was inserted 3' to the somatic ACE transcriptional start site but 5' to somatic ACE exon 1. Next, a 2.3-kb albumin promoter/enhancer was placed immediately 3' to the neomycin cassette. The albumin promoter/enhancer, termed NB, was a gift of Dr Richard Palmiter (University of Washington). Animal care was supervised by the Emory University Division of Animal Research and followed accepted practices of the American Veterinary Medical Association.

The targeting construct was linearized and electroporated into R1 ES cells. These cells were derived from a 129/SV×129/SvJ F1 embryo.<sup>6</sup> After positive and negative selection, individual ES cell clones were isolated and screened for targeted homologous recombination using a strategy of polymerase chain reaction (PCR) and genomic Southern blot analysis. The generation of chimeric, heterozygote, and homozygous mutant mice was performed as previously described.<sup>2,3</sup> Blastocysts were obtained from C57Bl/6 mice. Chimeric mice were mated to C57Bl/6 mice. All studies were performed on F2 generation litters generated from the breeding of F1 heterozygous animals (ACE<sup>+/-</sup>).

### Genotyping

PCR genotyping of genomic DNA relied on 2 separate reactions to amplify a wild-type-specific and a mutant-specific fragment. The wild-type reaction used a primer pair flanking the site of insertion of the 3.1-kb neomycin and the 2.3-kb albumin promoter cassettes. The primers 5'-ACTTTGGAGCGAGGAGGAAGC-3' and 5'-AACAGCACCAGCAGCGACAGCATCAA-3' yielded a 213-bp product from wild-type genomic DNA. To detect the mutant allele, a forward primer (5'-CTCTACAGATTAATTCAGTACAG-3') from the neomycin cassette was paired with a reverse primer from

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the albumin promoter (5'-AAGTGAATGAGCAAGCAGAA-3'). These amplified a fragment of approximately 500 bp.

For Southern blot analysis, DNA was digested to completion with the restriction enzyme *Tth111I* and was probed with a 1.1-kb genomic ACE fragment from the region immediately 3' to the targeting construct. This probe hybridizes to the ACE gene but not to the targeting construct. The mutant allele gave rise to a band of approximately 10 kb, whereas the wild-type allele gave rise to a band of >20 kb.

### ACE Activity

ACE activity was measured using the ACE-REA kit from American Laboratory Products Company, Ltd (ALPCO). ACE activity was defined as that inhibitable by lisinopril. For the determination of tissue ACE activity, mice were euthanized under anesthesia and their organs were weighed and frozen in liquid nitrogen. Later, each organ was gently homogenized in ACE homogenization buffer (50 mmol HEPES, pH 7.4, 150 mmol NaCl, 25  $\mu$ mol ZnCl<sub>2</sub>, and 1 mmol AEBSF) lacking detergent. These homogenates were centrifuged at 10 000g and the supernatant discarded. The pellets were resuspended in ACE homogenization buffer containing 0.5% Triton X-100 and vigorously rehomogenized. Total organ ACE was then calculated using the known weight of the whole organ. Total plasma volume was estimated as 26.6  $\mu$ L per gram of animal.<sup>7</sup>

### Western Blotting and Immunohistochemistry

Samples for Western blotting were homogenized in ACE homogenization buffer containing 0.5% Triton X-100. Equivalent amounts of protein were then separated by SDS-PAGE on a 10% gel. The membranes were probed with a 1:250 dilution of a rabbit polyclonal anti-mouse ACE antibody and exposed to film using enhanced chemiluminescence.

Tissue samples were taken at euthanasia and preserved either in Bouin's fixative or 10% buffered formalin. They were then embedded in paraffin and prepared for immunohistochemistry using standard techniques. ACE was detected using a rabbit polyclonal anti-mouse ACE antibody diluted 1:400 and immunoperoxidase as previously described.<sup>8</sup>

### Plasma Angiotensin I and II

Mice were exsanguinated by cardiac puncture, and blood was collected on ice in tubes containing 1.6 mg/mL potassium-EDTA, 100  $\mu$ mol/L amastatin, 100  $\mu$ mol/L bestatin, and 4  $\mu$ g/mL lisinopril.<sup>7</sup> Plasma was immediately frozen and stored at -80°C. Angiotensin I and II levels were measured by radioimmunoassay as previously described.<sup>9</sup> The assay background was determined by measuring peptide levels in angiotensinogen knockout mice, animals genetically modified to lack angiotensin I and angiotensin II. Background values were subtracted from the ACE.3 measurements to obtain the final data.

### Blood Pressure

Systolic blood pressure was measured using a Visitech Systems BP2000 automated tail cuff system as previously described.<sup>2</sup> To measure the blood pressure response to captopril, a baseline blood pressure (day 0) was determined for each mouse. At the end of day 0, captopril was added to the drinking water at an appropriate concentration to deliver 50 mg/kg of drug each day to each mouse. For each of the next 5 days, 25 measurements of systolic blood pressure were recorded for each animal. The first 5 were discarded and the next 20 were averaged. Fresh captopril solution was added at the end of days 2 and 4. At the end of day 5, the captopril was replaced with tap water, and blood pressure measurements were continued for 4 subsequent days.

### Hematocrit and Urine Collection

To measure hematocrit, mice were bled from the tail vein and blood collected in microcapillary tubes. These were centrifuged for 4 minutes at 12 000g and read in a manual microcapillary reader. For urine collection, littermate mice were first deprived of water in their

cages for 6 hours. They were then kept without food or water for 24 hours in individual metabolic chambers that separated urine from feces. Mineral oil was placed in the urine collection vial to minimize evaporation. At the end of the 24-hour collection period, the total urine output was measured and urine osmolality determined using a Wescat 5500 Vapor Pressure Osmometer (Wescor Inc).

## Results

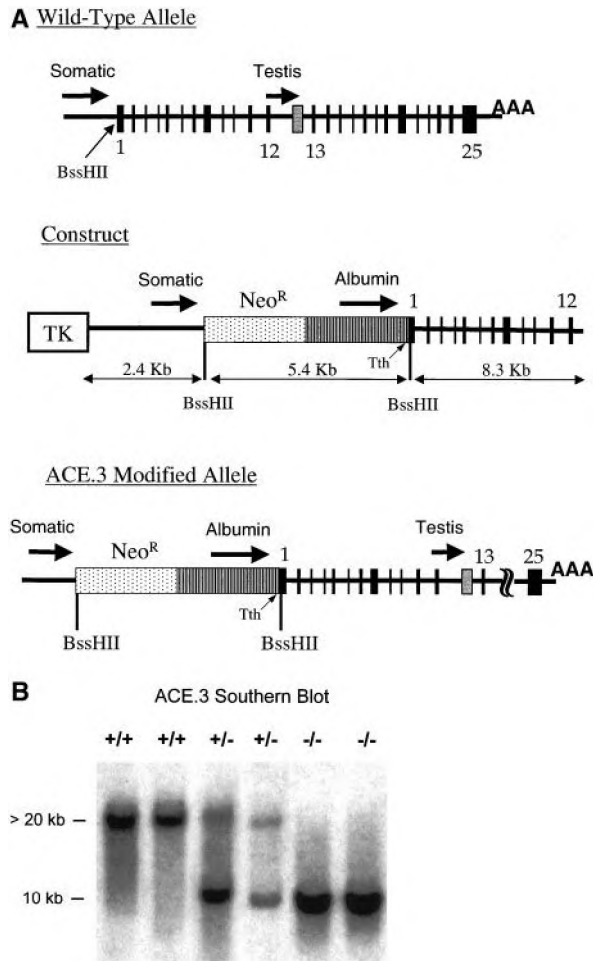
To create mice that only express ACE within the liver, we made a targeting construct in which a neomycin resistance cassette (KT3NP4) and an albumin promoter/enhancer were inserted between the transcription and the translation start sites for somatic ACE (Figure 1A).<sup>10,11</sup> The neomycin cassette contains the antibiotic resistance gene and exon 9 from the mouse hypoxanthine phosphoribosyltransferase gene. This exon encodes a strong transcriptional termination signal and was oriented to terminate transcription initiated by the somatic ACE promoter. Thus, the targeting construct is designed to substitute control of somatic ACE transcription from the somatic ACE promoter to the albumin promoter.

Mice homozygous for the ACE.3 mutation (-/-, knockout) were created using targeted homologous recombination in embryonic stem cells. Proper homologous recombination was identified using a combined strategy of PCR and genomic Southern blot analysis (Figure 1B). ACE.3<sup>-/-</sup> mice have normal serum electrolytes including normal serum potassium and creatinine levels. They show a very mild elevation of liver-derived serum transaminases (serum glutamic-oxaloacetic transaminase [U/L]: wild-type 67 $\pm$ 5, heterozygous 85 $\pm$ 10, and knockout 118 $\pm$ 20; serum glutamate pyruvate transaminase [U/L]: wild-type 29 $\pm$ 3, heterozygous 27 $\pm$ 4, and knockout 47 $\pm$ 8) (n $\geq$ 5). However, as discussed below, the knockout animals have a normal liver histology.

### Tissue Distribution of ACE

To evaluate the change in tissue ACE distribution, age matched ACE.3<sup>+/+</sup> and ACE.3<sup>-/-</sup> mice were euthanized, and tissue homogenates were analyzed by Western blot using a rabbit polyclonal anti-mouse ACE antibody (Figure 2A). A wild-type mouse produces very little ACE within the liver. In contrast, the ACE.3<sup>-/-</sup> mice demonstrate significant hepatic ACE expression. A wild-type mouse contains an enormous amount of ACE in the lung due to the high content of endothelium within this organ. In comparison, the ACE.3<sup>-/-</sup> mice have virtually no ACE expression within the lung. Indeed, ACE.3<sup>-/-</sup> mice showed no ACE in the heart, aorta, intestine, striated muscle, and seminal vesicles, all organs in which ACE.3<sup>+/+</sup> mice have easily identifiable levels of ACE. As anticipated, testis ACE production was not altered in the ACE.3<sup>-/-</sup> mice because this isozyme is due to an intragenic promoter unmodified by the targeting construct. In contrast, the production of ACE by somatic tissues (Leydig and endothelial cells) within the testis was not observed in the ACE.3<sup>-/-</sup> mice.

To quantitate organ specific ACE expression, a large cohort of animals was euthanized, and individual organs were homogenized using a protocol that permitted evaluation of tissue ACE activity in a preparation relatively free of plasma contamination. Total organ ACE activity, normalized for the



**Figure 1.** ACE.3 targeting construct. A, Top, Wild-type organization of the ACE locus. Both the somatic ACE promoter and the testis ACE promoter are indicated with arrows. A unique *BssHII* restriction site is located between the transcription start site and the translation start site of somatic ACE. The start site for testis ACE transcription is indicated by a box between the 12th and 13th exons of somatic ACE. The targeting construct used for homologous recombination (middle) contained 10.7 kb of homologous genomic DNA organized into left and right arms of 2.4 and 8.3 kb. Between these arms, we incorporated the 3.1-kb KT3NP4 neomycin resistance cassette (*Neo<sup>R</sup>*) and the 2.3-kb albumin promoter such that the structural portion of the ACE gene would now be under the control of the albumin promoter. Effects of the somatic ACE promoter were minimized by positioning the neomycin cassette such that any transcripts generated by this promoter would terminate within the neomycin construct. A thymidine kinase (*TK*) cassette was positioned 5' to the 2.4-kb homology arm. With proper homologous recombination, this will not be incorporated within the modified ACE allele (bottom). A unique *Tth*1111 restriction site within the targeting construct is indicated by *Tth*. B, Southern blot analysis. Genomic DNA from wild-type (+/+), heterozygous (+/-), and ACE.3 knockout (-/-) mice was digested with *Tth*1111 and was probed with a 1.1-kb genomic ACE fragment from the region immediately 3' to the targeting construct. This probe hybridizes to the ACE gene but not to the targeting construct. The mutant allele gave rise to a band of approximately 10 kb, whereas the wild-type allele gave rise to a band of >20 kb.

weight of individual animals, was then calculated for each genotype (Figure 2B). Although ACE.3<sup>-/-</sup> mice have internal organs equivalent in weight to littermate wild-type mice, there is a marked change in the organ distribution of ACE

activity. ACE.3<sup>-/-</sup> mice have approximately 87-fold more hepatic ACE activity than the ACE activity present in the livers of wild-type mice. The opposite was true for lung where no ACE activity was detected in the lung homogenates of the ACE.3<sup>-/-</sup> mice. Evaluation of ACE activity in ACE.3<sup>-/-</sup> kidney showed levels approximately 14% that observed in the kidneys of wild-type mice. When evaluated as activity per  $\mu$ g of solubilized protein, this figure was 12.5% of wild-type. Other somatic tissues, including the aorta, heart, and small intestine, showed a complete lack of ACE activity in ACE.3<sup>-/-</sup> mice (data not shown). ACE.3<sup>-/-</sup> mice have approximately 80% the plasma ACE activity of wild-type mice. Total plasma ACE activity, per animal, was estimated to be about 11% the total ACE activity present in liver. We hypothesize that the plasma ACE present in ACE.3<sup>-/-</sup> mice results from the proteolytic release of ACE from the surface of hepatocytes.

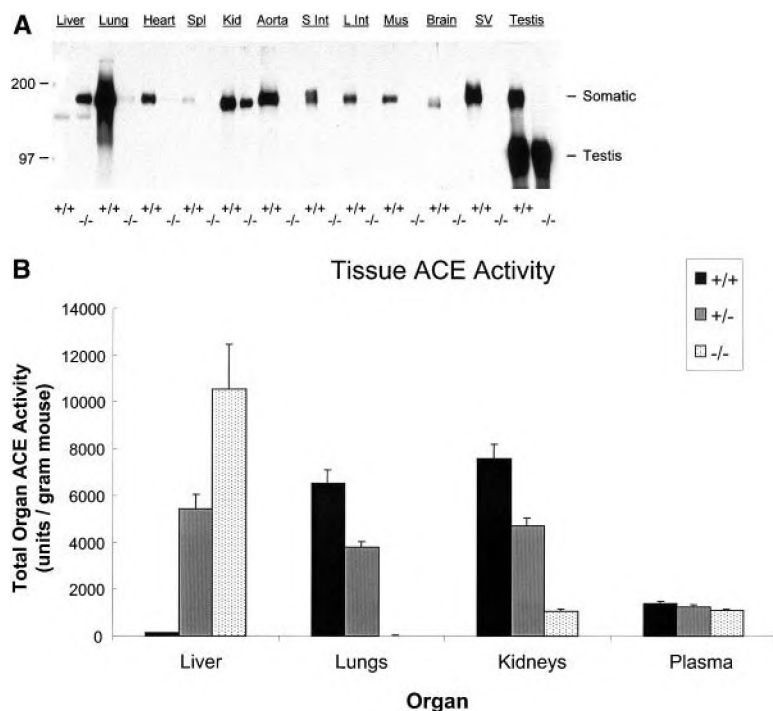
Immunohistochemistry was used to evaluate the tissue patterns of ACE expression in the liver, lung, and kidney of ACE.3 mice (Figure 3). Although livers from wild-type animals show very little ACE expression (Figure 3A), abundant immunostaining was present in the cell membranes of hepatocytes from the ACE.3 knockout mice (Figure 3B). In contrast, the lungs of the ACE.3<sup>-/-</sup> mice lacked any staining for ACE, whereas abundant ACE expression was evident in its wild-type counterpart (Figures 3C and 3D). Figures 3E and 3F show representative staining from the kidneys of ACE.3 wild-type and ACE.3<sup>-/-</sup> animals. Whereas the kidneys of the wild-type mouse showed intense staining for ACE in the straight portion of the proximal tubule (predominantly the S3 segment), equivalent epithelium in ACE.3<sup>-/-</sup> mice showed markedly reduced levels of staining. Instead, the ACE.3<sup>-/-</sup> kidneys showed a very low level of ACE expression throughout the proximal tubule.

We also carefully evaluated the kidneys of ACE.3<sup>-/-</sup> mice to assess whether residual ACE activity was due, in part, to ACE expression by renal blood vessels. For this study, portions of kidney from both wild-type and ACE.3<sup>-/-</sup> mice were incorporated into the same paraffin blocks and were processed on the same slides. As expected, the vascular endothelium of wild-type mice was intensely positive for ACE expression (Figure 3G, arrow). In contrast, endothelium from blood vessels of ACE.3<sup>-/-</sup> mice exhibited no such ACE expression (Figure 3H). Vascular adventitia showed a similar pattern with staining for ACE in wild-type blood vessels but none in the ACE.3<sup>-/-</sup> mice.

### Physiological Analysis

Next, we measured systolic blood pressure to determine whether the ACE expression in ACE.3<sup>-/-</sup> mice could compensate for the lack of endothelial expression in these animals (Figure 4A). Pressure was determined using a computerized tailcuff manometer after extensive training of the animals.<sup>12</sup> A cohort of 48 littermate ACE.3 mice showed no differences in blood pressure between wild-type, heterozygous, and ACE.3<sup>-/-</sup> mice. Thus, ACE.3<sup>-/-</sup> mice, while lacking endothelial ACE, have no difficulty maintaining a normal systolic blood pressure. Although it is currently impossible to quantify the contribution of residual proximal tubular ACE to the





**Figure 2.** Tissue ACE expression. A, ACE.3 wild-type (+/+) and knockout (-/-) mice were euthanized, and organ extracts were analyzed by Western blot using a rabbit anti-mouse ACE antibody. Somatic ACE is detected as a protein of approximately 170 kDa, whereas testis ACE is a more diffuse band centered around 95 kDa. The organs studied were liver, lung, heart, spleen (spl), kidney (kid), aorta, small intestine (S Int), large intestine (L Int), striated muscle (Mus), brain, seminal vesicles (SV), and testis. Wild-type mice have little ACE in the liver but abundant ACE in the lung. In contrast, the ACE.3<sup>-/-</sup> mice have significant liver ACE but virtually no ACE protein in the lung, aorta, heart, gut, muscle, and seminal vesicles. The kidney of ACE.3<sup>-/-</sup> mice does have residual somatic ACE expression. The testis of ACE.3<sup>-/-</sup> mice expressed no somatic ACE but continued to produce abundant testis ACE. B, ACE.3 wild-type (black), heterozygote (gray), and knockout (stippled) mice were euthanized and ACE activity measured in organ homogenates. Total organ ACE activity was then calculated for the liver, lungs, kidneys, and plasma of each mouse and normalized for the mass of the mouse. The livers of ACE.3<sup>-/-</sup> mice have abundant total ACE activity, due in part to the large size of this organ. In contrast, ACE.3<sup>-/-</sup> mice have no detectable ACE activity in lung. ACE.3<sup>-/-</sup> mice have about 14% of the renal ACE found in wild-type mice. The data are the average of 5 or 6 individual mice with these exceptions: 11 mice for the +/+ and -/- kidney, 16 to 18 mice for each plasma ACE genotype. All data presented are the group mean  $\pm$  SEM.

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blood pressure regulation of the ACE.3<sup>-/-</sup> mice, it is clear that endothelial ACE is not required for this precise control.

In order to get mechanistic insight into the blood pressure observed in the ACE.3<sup>-/-</sup> mice, we investigated plasma angiotensin peptide levels. This study showed no significant difference in plasma angiotensin I or angiotensin II levels in the ACE.3<sup>-/-</sup> mice as compared with littermate wild-type mice. For example, angiotensin II levels in the plasma of ACE.3<sup>-/-</sup> mice averaged  $172.4 \pm 17.6$  pg/mL (n=12) as compared with  $139.8 \pm 23.2$  pg/mL in ACE.3<sup>+/+</sup> mice (n=13).

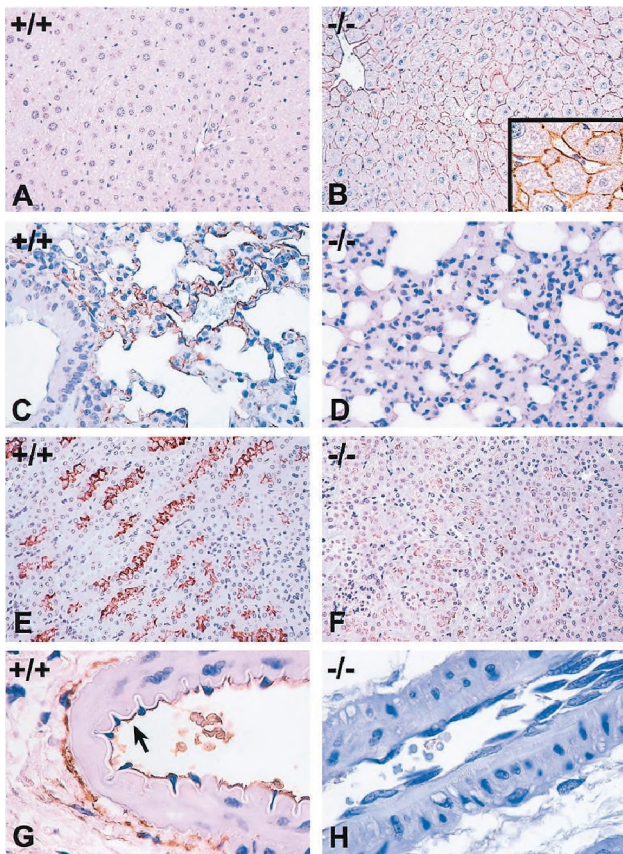
The finding that ACE.3<sup>-/-</sup> mice have normal plasma levels of angiotensin II and a normal blood pressure suggests that ectopic expression of ACE within the liver provides sufficient enzymatic activity to allow physiologically appropriate production of angiotensin II. This hypothesis predicts that ACE.3<sup>-/-</sup> animals should respond to ACE inhibitors with a reduction of blood pressure. To test this, a cohort of ACE.3<sup>-/-</sup> mice and littermate wild-type mice were treated with the ACE inhibitor captopril for 5 days (Figure 4B). Blood pressure was measured in these animals before, during, and after captopril administration. This study showed no difference in the response of wild-type and ACE.3<sup>-/-</sup> mice to the administration of an ACE inhibitor. On cessation of captopril, the blood pressures of both groups of animals rapidly returned to control levels. This experiment showed that the normal blood pressure observed in ACE.3<sup>-/-</sup> mice is directly dependent on the ACE activity present in these animals.

Dehydration is associated with an activation of the renin-angiotensin system. To investigate if ACE.3<sup>-/-</sup> mice were able to tolerate dehydration, 6 knockout mice and 6 wild-type mice were not allowed water for 24 hours. Blood pressure, heart rate, and body weight were measured at 12, 16, 20, and

24 hours of water deprivation. No statistically significant differences were observed between wild-type and ACE.3<sup>-/-</sup> mice during the course of the dehydration. For example, 24 hours of dehydration produced no statistical reduction of systolic blood pressure in either wild-type or ACE.3<sup>-/-</sup> mice. Also, both groups lost an equivalent percentage of body weight (wild-type,  $9.3 \pm 0.7\%$  versus ACE.3<sup>-/-</sup>,  $9.8 \pm 1.3\%$ ). Thus, ACE.3<sup>-/-</sup> mice tolerate dehydration for 24 hours in a fashion seemingly equivalent to wild-type mice.

Mice null for all ACE expression produce large amounts of a dilute urine.<sup>1,2</sup> In part, this is due to underdevelopment of the renal medulla and papilla, a finding also observed in angiotensinogen and AT<sub>1</sub>-receptor knockout mice.<sup>13-16</sup> To study the renal concentrating ability of ACE.3<sup>-/-</sup> mice, these animals were water deprived and placed in metabolic cages for a 24-hour quantitation of urinary volume and urine osmolality. Twelve wild-type (+/+) mice, 11 +/- mice, and 13 -/- mice produced an average volume of  $403 \pm 64$ ,  $473 \pm 93$ , and  $403 \pm 100$   $\mu$ L of urine, respectively, whereas the osmolality of the urine was  $2162 \pm 253$  (+/+),  $2080 \pm 213$  (+/-), and  $2373 \pm 252$  (-/-) mOsm/kg H<sub>2</sub>O. This experiment showed that ACE.3<sup>-/-</sup> mice concentrated urine in a fashion equivalent to that of wild-type mice. Indeed, evaluation of ACE.3<sup>-/-</sup> mice has verified that they can concentrate to greater than 3000 mOsm/kg H<sub>2</sub>O when a drop of urine is examined after 24 hours of water deprivation. Histological examination of the kidneys from ACE.3<sup>-/-</sup> mice showed no anatomic lesions. ACE.3<sup>-/-</sup> mice have a serum creatinine and blood urea nitrogen equivalent to that of wild-type mice.

An unexpected finding noticed in ACE knockout mice was the presence of anemia.<sup>7</sup> Examination of ACE.3<sup>-/-</sup> mice showed a hematocrit of  $52 \pm 0.5\%$  (n=17), which was equiv-



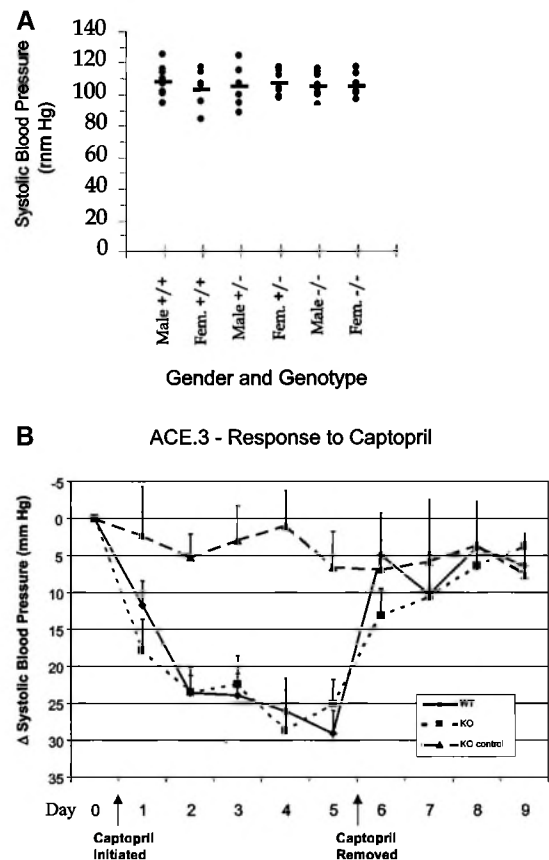
**Figure 3.** ACE immunohistochemistry. Tissues from wild-type (+/+) and ACE.3 knockout (-/-) mice were prepared and stained with rabbit anti-ACE antibody. A and B, Sections of liver. The high-power insert in B shows abundant ACE within the cell membranes of hepatocytes. Despite this, the overall histological appearance of ACE.3<sup>-/-</sup> liver was unchanged from that of wild-type liver. C and D, Sections of lung. Wild-type mice have abundant pulmonary ACE. In contrast, the lung from ACE.3<sup>-/-</sup> mice has no detectable ACE. E and F, Sections of kidney. Wild-type kidney has abundant ACE in proximal tubular epithelium. In contrast, ACE.3<sup>-/-</sup> mice show much reduced levels of ACE staining within this same tissue. Within the kidney, arterioles from wild-type mice have easily observable endothelial and adventitial immunoreactivity for ACE (G, arrow indicates endothelium). In contrast, renal arterioles from ACE.3<sup>-/-</sup> mice (H) show no such staining. In fact, no endothelial immunoreactivity for ACE was present anywhere in ACE.3<sup>-/-</sup> mice. A and B were photographed with a 10× objective; C through F, 20× objective; G, H, and B insert, 100× oil objective.

alent to the hematocrit of  $52 \pm 0.7\%$  ( $n=16$ ) measured in littermate wild-type mice.

### Discussion

One of the goals of this study was to develop a method allowing selective tissue expression of ACE. Ultimately, we wish to create lines of mice expressing ACE in a variety of individual tissues in the absence of generalized tissue expression. Our first attempt was to make a mouse with expression of ACE limited to the liver. The availability of the albumin promoter, the very low endogenous levels of ACE expression by the liver, and the large size of this organ made this an attractive model. Although there are several potential approaches to creating liver-specific protein expression, our

approach was to use homologous recombination to substitute promoter control of the somatic ACE gene from the endogenous promoter to the inserted albumin promoter. We hypothesized that such a mouse would express ACE in the liver but not in vascular endothelium. For the most part, this approach proved successful. ACE.3<sup>-/-</sup> mice have abundant hepatic expression of ACE while lacking ACE expression by endothelium, adventitia, the gut, and several other tissues that normally make this protein. Only in the kidney do we see residual somatic ACE expression, and even here, careful quantitation showed that ACE.3<sup>-/-</sup> mice made only 12% to 14% of the renal ACE found in wild-type mice. This residual ACE expression is probably due to the specificity of the



**Figure 4.** Systolic blood pressure. A, Basal systolic blood pressure was determined for a cohort of 48 ACE.3 littermate mice over 4 consecutive days using an automated tail-cuff manometer. The systolic blood pressure for each mouse (●) is the average of 80 individual measurements. A solid bar (■) represents the average for each group of 8 mice. The systolic blood pressure is not different between wild-type (+/+), heterozygous (+/-), and knockout (-/-) mice of either gender. B, Baseline blood pressure was established for a cohort of ACE.3 animals. Captopril was then added to the drinking water of 6 +/+ (●) and 6 -/- (■) mice, and systolic blood pressures were monitored daily. After 5 days, the captopril was discontinued with blood pressure measurements continuing for 4 additional days. During the 9 day experiment, the blood pressure was also monitored for a control group of 4 ACE.3<sup>-/-</sup> mice (▲) that never received captopril. In response to captopril, the systolic blood pressures of ACE.3<sup>+/+</sup> and ACE.3<sup>-/-</sup> mice decreased in a similar fashion, both in magnitude and duration. Likewise, mice of both genotypes exhibited a rapid rebound in blood pressure once captopril was discontinued.



albumin promoter in that the original characterization of albumin promoter cassettes similar to that used in the ACE.3<sup>-/-</sup> mice showed low levels of reporter gene expression within the kidney.<sup>11</sup>

In the ACE.3<sup>-/-</sup> model, the total body load of ACE is substantial. In a sense, the liver of ACE.3<sup>-/-</sup> mice approximates the function of the lung in wild-type animals, allowing physiological regulation of the renin-angiotensin system in ACE.3<sup>-/-</sup> mice to generate appropriate levels of angiotensin II. This, in turn, results in a mouse with a normal blood pressure and normal renal function.

So what of endothelial ACE? In a wild-type mouse, and in a human, endothelium provides a major source of tissue ACE. Indeed, studies by Ng and Vane<sup>17</sup> showed sufficient ACE in the lung to entirely convert blood angiotensin I to angiotensin II during a single transit. What we now observe in ACE.3<sup>-/-</sup> mice is that, surprisingly, endothelial expression of ACE is not obligatory for basal blood pressure regulation; in these mice, sufficient ACE expression by a different tissue source can compensate for the lack of endothelial ACE. However, we must offer a caveat. Current hypotheses concerning the renin-angiotensin system implicate the local generation of angiotensin II in some of the physiological and pathophysiological effects of this peptide, including aspects of cardiovascular and renal injury.<sup>5</sup> In this study, we made no attempt to quantitate whether ACE expression in ACE.3<sup>-/-</sup> mice is sufficient for physiological regulation in the face of any form of tissue injury. Indeed, a powerful use of ACE.3<sup>-/-</sup> mice will be to test various models of injury to assess the role of endothelial ACE expression. Such studies may reveal specific and unique requirements for endothelial ACE expression, but under basal conditions, the expression of ACE by the liver is capable of substituting for endothelial ACE expression in that ACE.3<sup>-/-</sup> mice have a normal blood pressure and normal renal function.

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